

Heartwood Formation in Living Stumps of Douglas-Fir

By R. W. HEMINGWAY and W. E. HILLIS*

Physiology and Microstructure Section, Division of Forest Products, C.S.I.R.O. Melbourne, Australia

Summary

An anatomical and chemical examination was made of living stumps of Douglas-fir. Changes in heartwood and extractives formation are not significant under the conditions of severe physiological stress that existed unless cell morphology was also altered. It is proposed that the factors controlling the amount and composition of heartwood extractives are incorporated in the ray cells during the early stages of their development.

Introduction

The width of sapwood and the extractives content of heartwood generally varies throughout a tree and between different trees of the same species. Factors reputedly responsible for these variations include hormonal influence, water stress and other environmental and genetic effects. BORMANN [1962] demonstrated that exchange of metabolites between trees through root grafts is a common occurrence. These grafts enabled the stumps of trees to continue to live after the loss of their green tops. It appeared to us that such stumps provided a combination of factors related to heartwood formation. It might be expected that living stumps would receive only the excess nutrients of the host tree and the living stump may be comparable to an intact tree under severe physiological stress. LANNER [1961] reported the formation of heartwood in stump tissues of Douglas-fir, true firs and pines. There are at least four zones in such stumps; the heartwood of the tree (normal heartwood and normal cell development), the tree sapwood which can be changed to heartwood after the tree is felled (normal cell development but abnormal heartwood) and the surrounding stump tissue the interior of which is sometimes converted to heartwood (abnormal heartwood and abnormal cell development). This paper reports an examination of living stumps of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) with reference to heartwood formation.

* The authors are particularly grateful to Mr. J. WALTERS, Director of the Research Forest and to Professor J. A. F. GARDNER, Dean of the Faculty of Forestry, of the University of British Columbia, Canada for their considerable help in the collection and transport of the samples of living stumps which formed the basis of this investigation. They are also grateful to Mr. A. CESELLI, Mrs. J. JURICKAY and Miss D. MUSTON for assistance in the anatomical studies.

Results

Description of Living Stumps

Callus tissue completely or partly covered the top of the stumps which were collected from sheltered positions in the Haney Forest of the University of British Columbia. Removal of the top 1 to 2 inches of the stumps revealed that most of the stumps had some decay and that the wood which formed after the trees were felled (stump wood) completely surrounded the tree wood. Growth ring counts showed that the trees were aged 45 to 73 years at time of felling with diameters of 33 to 42 cm. The growth rings in the stump wood were less distinct than those in the tree wood but corresponded within 3 years of the 20 years that had elapsed since felling. The width of the most recently formed growth rings became increasingly narrow (Fig. 1). The width of the stump wood varied from 3 to 25 mm in different parts of the stumps. Wide stumpwood zones were presumably associated with proximity to the root grafts. There were some zones of resin soaking in the region corresponding to the cambium at the time of felling, but in spite of this, the stump grew over these zones.

The stump wood usually contained both sapwood and heartwood, the latter being readily detected by having a redder colour than normal heartwood. The width of stump heartwood was variable. In locations where stumpwood was thin, stump sapwood and the outer tree sapwood were sometimes not transformed to heartwood. The zone which was sapwood (about 20 growth rings) when the tree was felled (tree sapwood) was sometimes blue stained and resin soaked. In some cases there were blue stained-resin soaked patches in the inner portions and normal transformation to pink colored heartwood in the outer layers of the tree sapwood. Resin soaked patches were also observed in the tree heartwood. The color of these patches was similar to that of the surrounding heartwood indicating that resin soaking took place after polyphenol formation in contrast to the tree sapwood where apparently resin soaking occurred first. It was difficult to obtain a radial strip containing all four zones but without blue staining and resin soaking in some portion.

Anatomical Comparison of Stump Wood with Tree Wood

Early and late wood bands were evident in the growth rings of Douglas-fir stump wood although LANNER [1961] reported lack of contrast in *Pinus taeda*. Examination of Douglas-fir cross sections indicated less latewood and a less abrupt transition from early to latewood in the stump wood when compared with tree wood. Most of the tangential sections of Douglas-fir stump wood showed severe disorientation of tracheid direction (Fig. 2). SCHULTZ and WOODS [1967] reported that the xylem tissue in the living stumps of *P. taeda* also showed orientation in more than one direction and the tracheids appeared to be very short and irregular in shape. In *P. taeda* the stump wood tracheids were one-fifth that of normal tracheids. We found a large proportion of long tracheids after maceration of Douglas-fir stump wood. The tracheids obtained from severely disorientated samples assumed a normal straight shape after suspension in water but a high proportion of broken fibres was observed. The average unbroken fibre length of Douglas-fir stump wood was 2.8 mm compared to 3.5 mm in adjacent tree wood.

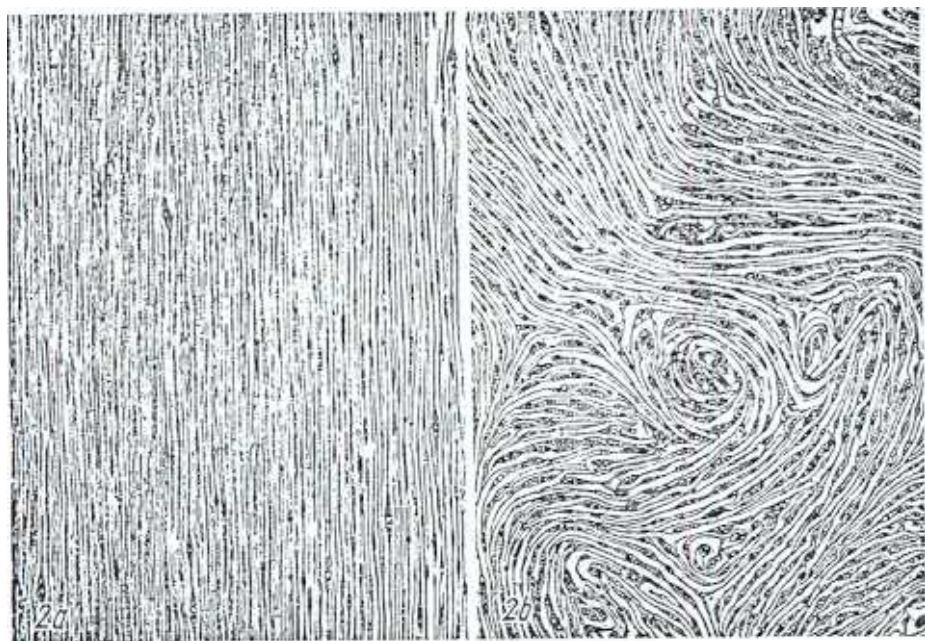
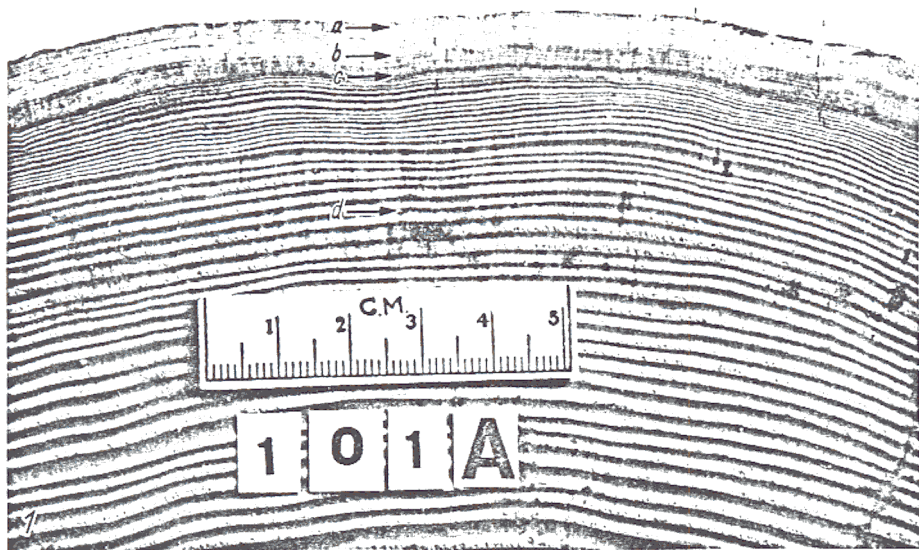


Fig. 1. Cross section of living stump of Douglas-fir showing various zones of heartwood formation; a-b is stump sapwood, b-c is stump heartwood, c-d is tree sapwood, d to pith is tree heartwood. Magnification 25 ×

Fig. 2. Tangential sections of tree wood (a) and stump wood (b) showing distortion of fiber direction and unusual ray structure in stump wood. Magnification 25 ×

The average specific gravity of stump wood was essentially the same as tree wood (ca. 0.41 g/cm³). However, microscopic examination of stump wood revealed more thin walled cells than was observed in tree wood. There was a decrease in specific gravity from inner stump wood to the bark. The ray volume of stump wood was significantly greater than in tree wood (13.3 compared to 9.9%) and this was associated with a higher proportion of multi-seriate rays, although there appeared to be a slight increase in the number of individual rays per unit area (Table 1).

Table 1. Ray distribution of stump wood and tree wood

	Stump Wood ¹	Tree Wood ¹
Ray volume %	13.3	9.9
Number of cells/ray	6.8	5.9
Number of rays/area	41.6	38.3

¹ Average of 12 samples.

Table 2. Amounts of extractives in stump wood compared with adjacent tree wood

	Petroleum solubles ¹ %	Alcohol solubles ¹ %
Stump sapwood	2.2	4.0
Stump heartwood	5.8	6.6
Tree sapwood	4.4	3.5
Tree heartwood	2.7	3.0

¹ Average of 4 samples.

Extractives

General

A comparison of the amounts of petroleum and the subsequent alcohol-soluble material in the stump sap- and heartwood and tree sap- and heartwood indicated that the stump heartwood contained significantly larger amounts of both classes of extractives than the other zones (Table 2). The amount of petroleum soluble materials in different samples of the stump sapwood and heartwood were close to other reported values. Figures between 2 and 5% of alcohol-benzene solubles have been previously recorded for sapwood and heartwood respectively [CAMPBELL, SWAN, WILSON 1965]. There was considerable variation in the amount of these extractives in both tree sapwood and heartwood. Patches of resin soaked tree wood contained up to 30% petroleum soluble extracts whereas the amount of them in the tree sapwood that was not obviously resin soaked varied between 1 and 10%.

The amount of alcohol soluble extracts in individual samples was similar to the average values (Table 2), except for the tree sapwood zone which was highly variable. In areas where there was little resin soaking the alcohol solubility was high (about 4%) while in resin soaked areas the alcohol solubility was low (1 to 2%). There appeared to be an inverse relationship between resin content and alcohol solubility. The alcohol solubility of both stump sapwood and heartwood was higher than that of tree wood.

Polyphenols

Paper chromatography of alcohol extracts from the various zones is summarized in Table 3. There was no significant qualitative difference between stump heartwood, tree sapwood or tree heartwood. Dihydroquercetin was by far the major compound in all samples except where compound "A" predominated. SQUIRE, SWAN and WILSON [1967] observed significant amounts of dihydrokaempferol

(0.3%), pinobanksin (0.1%) and quercetin (0.1%) along with dihydroquercetin (1.0%). In our work, dihydrokaempferol was observed in more than trace amounts only in inner tree heartwood. Significant spots for pinobanksin and quercetin were not observed despite considerable overloading of the dihydroquercetin spot.

Table 3. *Composition of polyphenolic extractives in Douglas-fir tissues*

Compound ¹	Stump wood		Tree wood		<i>R_f</i>	Description ²	
	Sap	Heart	Sap	Heart		Fluorescence	pNA
DHQ	+	++	+	++	0.8/0.3	—	tan
A	++	—	—	—	0.4/0.3	—	tan-yellow
B	+	—	—	—	0.4/0.5	—	pink
C	—	+	+	+	0.7/0.4	blue-green	nil
D	—	+	+	+	0.6/0.8	—	tan
E	—	+	+	+	0.7/0.5	—	yellow
F	+	++	+	+	0.7/0.9	blue-green	nil
G	+	++	+	+	streak/0		

¹ DHQ = dihydroquercetin; A is probably a DHQ glycoside.

² *R_f* in butanol-acetic acid-water (6:1:2) in one direction then 2% acetic acid followed by butanol-ammonia-water (20:3:10). pNA = diazotised *p*-nitroaniline.

It is significant that compound "A", possibly dihydroquercetin-3'-glucoside, and compound "B", probably another glycoside, were not found in tree sapwood even though all this zone had not changed to heartwood as judged by its coloration. HERGERT and GOLDSCHMID [1958] have reported the disappearance of dihydroquercetin-3'-glucoside when Douglas-fir heartwood is formed. The alcohol soluble extract from stump heartwood was a darker red color than the other extracts. This color was removed by passing the extract over a short polyamide column and observation of paper chromatograms indicated that this column largely removed the polymer streak. Stump heartwood appears to contain considerably more of a highly colored polymer than normal Douglas-fir heartwood.

Quantitative Aspects

The amount of dihydroquercetin in a radial strip from a stump was determined by gas liquid chromatography of the trimethylsilyl ether. Although the amount of alcohol solubles in the stump heartwood was significantly higher than that of normal heartwood, the amount of dihydroquercetin in the two zones was essentially the same (Table 4). The amounts are within the range 0.90 to 1.8% previously reported [GARDNER, BARTON 1960; SQUIRE, SWAN, WILSON 1967; HANCOCK 1957; KENNEDY, WILSON 1956].

Stump sapwood contained a comparatively high concentration of dihydroquercetin (ca. 0.37%), but other workers [GARDNER, BARTON 1960; KENNEDY, WILSON 1956; HANCOCK 1957] have found that the sapwood in normal trees contains between 0.15 to 0.56%. The amount of dihydroquercetin in the outer tree sapwood of the stump was lower than that expected as this region has essentially the same color as normal tree heartwood. It appears that the pink color is not necessarily related to the amount of dihydroquercetin present. The amount of dihydroquercetin found in the middle and inner tree sapwood was very low and

corresponds with normal sapwood concentrations. The inner tree sapwood also contained large amounts of resin and some blue stain. The results indicated that resin soaking, which was associated with development of blue stain, inhibited the formation of polyphenols in some zones of the tree sapwood.

Table 4. *The dihydroquercetin and petroleum soluble contents in a radial strip from a living stump*

Tissue	Petroleum solubles % ±	Dihydro- quercetin % ±
Stump sapwood	2.8	0.37
Stump heartwood	5.9	1.13
Outer Tree Sapwood	1.4	0.35
Middle Tree Sapwood	1.5	0.11
Inner Tree Sapwood	10.6	0.14
Outer Tree Heartwood	8.1	1.07
Middle Tree Heartwood	8.4	1.25
Inner Tree Heartwood	13.3	0.68

¹ Average of 2 determinations.

² Average of 4 determinations.

Table 5. *Radial distribution of dihydroquercetin across a tree sapwood zone*

Growth ring Number	Colour	% Dihydro- quercetin ¹
3	white	0.27
6	white	0.22
13	pink	1.47
14	pink	1.23
18	pink	2.09
19	pink	0.53

Average of 4 determinations.

Because of the low dihydroquercetin content in the above sample of the tree sapwood zone, another radial strip, that appeared to be free from resin soaking, was analysed. No stump heartwood was evident in this sample. The growth rings number 3, 6, 13, 14, 18 and 19 were selected from the tree sapwood zone with growth ring number 1 corresponding to the last growth ring produced before felling. The amount of dihydroquercetin from these growth rings is given in Table 5. Low values obtained for growth rings number 3 and 6 suggest that heartwood formation had not occurred even though resin soaking had not taken place. The dihydroquercetin concentration in the other growth rings shows that dihydroquercetin formation takes place in amounts similar to or greater than its concentration in normal tree heartwood.

Resins

Resin acids in petroleum ether extracts were analysed by gas-liquid chromatography of their methyl esters. There was little difference in the resin acid composition of the various zones except for isomerization of levopimaric to dehydroabietic in the inner tree heartwood zones. The resin acid composition agreed with the results of ERDTMAN, KIMLAND, NORIN and DANIELS [1968] with the levopimaric-palustic peak being the largest with lesser amounts of dehydroabietic, abietic, neoabietic and isopimaric acids present. Small amounts of pimaric acid were also evident.

Discussion

At the present time, there is no decisive proof of the location of synthesis of extractives, or of the factors controlling the amounts of extractives deposited in heartwood. HERGERT and GOLDSCHMID [1958] have proposed that flavonoid

glycosides are synthesized in the leaves of Douglas-fir and that they are then transported down the phloem and radiate to the bark and heartwood boundary through the rays. ERDTMAN [1958] however has shown that, in pines, the bark polyphenols are primarily hydroxylated flavonoids while those in the heartwood are mainly stilbenes and flavonoids with fewer hydroxyls. Based on these constitutional differences ERDTMAN proposed synthesis of the bark polyphenols in the cork cambium and heartwood polyphenols in the xylem cambium. HILLIS [1968], on the other hand, after examination of the data from a number of trees, came to the conclusion that heartwood phenolic compounds are synthesized at the sapwood-heartwood boundary.

There are many factors possibly related to amounts of heartwood constituents formed. Availability of carbohydrate at the zone of synthesis would be expected to be an important parameter [HILLIS, HUMPHREYS, BAMBER, CARLE 1962]. Growth rate is another associated parameter which would be expected to reflect available carbohydrate at the xylem cambium. HILLIS [1968] has also suggested that the auxin-carbohydrate balance may be important to amounts of polyphenols formed.

The results of this study indicate that there was little difference between the polyphenol contents of tree heartwood and tree sapwood where resin soaking did not occur. When blue stain formation and resin soaking occurred they probably developed soon after wounding and prevented the synthesis of polyphenols in, or translocation to, some tree sapwood regions. It is possible however that resin soaking appeared because a lack of polyphenols allowed blue stain to develop which in turn facilitated resin soaking. The patchy distribution of resin soaking tree sapwood suggests that the first possibility is the most likely.

The slow growth rate of the stump wood which was of similar specific gravity to tree wood suggests that the amount of carbohydrate reaching the stump cambium was significantly reduced after crown removal. However in regions where resin soaking did not appear, tree sapwood contained normal amounts of dihydroquercetin. No direct evidence was obtained from the stumps regarding HERGERT and GOLDSCHMID's [1958] proposal that the extractives originate in the leaves and are translocated to the heartwood and bark. However, it is difficult to reconcile this view with the existence of 6-C-methyl flavonoids found in the Douglas-fir root bark [BARTON, 1967, 1969] but not elsewhere in the tree. There was no evidence of this compound or its derivatives in any xylem tissues of the stump showing that translocation of phenolic materials through the root grafts is unlikely. It would appear then that the formation of heartwood extractives was insensitive to changes in the amount of carbohydrate (as mentioned above) or flavonoid glycoside available at the cambium.

SQUIRE, SWAN and WILSON [1967] found most of the dihydroquercetin in the rays, but the increase in ray volume of the stump tissues has not resulted in a significant increase of dihydroquercetin. A significant difference observed in the cross sections of the living stumps was the high alcohol solubility of stump heartwood due largely to proportionately high levels of the colored polymer. This change did not occur until after crown removal when there were significant changes in cell morphology. It could be concluded from these results that the factors controlling the subsequent amount and composition of heartwood polyphenols formed are

incorporated in ray cells during the early stages of their development. Some support for this view is found in the observation that small amounts of dihydrokaempferol were found only in the inner tree heartwood. SQUIRE, SWAN and WILSON [1967] have demonstrated a strong seasonal variation of dihydroquercetin concentration within a growth increment that cannot be explained by variations in specific gravity. Since the heartwood-sapwood boundary does not follow annual rings, a consistent within-ring variation in dihydroquercetin should not be related to seasonal variation in translocation of flavonoid glycosides from leaves or the cambial zone. It would appear that within-ring variations of dihydroquercetin concentration may indicate differences in the ray cells across the growth ring rather than variations in flavonoid glycoside translocation to the sapwood-heartwood boundary. An earlier observation [KRAHMER, HEMINGWAY, HILLIS 1970] that different lignans can be found in cells that exist close together in heartwood also points to different metabolic pathways in individual ray cells.

The time period between development of the cells and formation of heartwood was little influenced by crown removal. WELLWOOD [1955] found that there was a gradual increase in the width of sapwood from the top of the stem to the bottom. This gradation may reflect greater auxin or carbohydrate depletion at increasing distance from the crown. The tree sapwood-heartwood boundary of the living stumps occurred at about 20 growth rings from the cambium at the time of crown removal and was fairly regular. The stump sapwood-heartwood boundary was more irregular both in terms of distance and growth increments from the cambium. In some locations the sapwood-heartwood boundary was still in the tree sapwood region (Table 5) while in other regions, heartwood was found in the inner 5 to 7 growth rings of the stumpwood. On an average our results indicated that heartwood development occurred close to the twenty year period found in the tree wood. Because of the slow and steadily decreasing growth rate of stump wood a sapwood zone 20 years wide represented proportionately much less wood than appeared in normal tree growth.

Experimental

Paper chromatograms were prepared using Whatman No. 2 papers developed in the first direction with butanol: acetic acid: water (6: 1: 2), and in the second with 2% acetic acid, after which the sheet was dried and again developed in the second direction with butanol-ammonia-water (20: 3: 10). The dried sheets were observed under ultraviolet light and sprayed with diazotised *p*-nitroaniline or a mixture of ferric chloride-potassium ferricyanide.

Dihydroquercetin was measured by gas-liquid chromatography of its trimethylsilyl ether prepared from pyridine extracts. TMS-dihydroquercetin was separated on 2% SE 30 on acid washed DMCS treated Chromosorb W (80 to 100 mesh) and peak areas compared to the internal standard TMS-*phloretin*. Oven temperature was programmed from 195° C to 215° C at 1° C/min. Carrier gas flow rate was 40 ml/min. The coefficient of variation for this method has been from 4 to 6%.

The petroleum extracts were separated on chromatoplates of Silica Gel GF 254 with thickness of 0.75 mm and developed with hexane-diethyl ether (85: 30). The appropriate zone was scraped from the plates, the free acids extracted with acetone, the extract evaporated and methylated with diazomethane. The methylated resin

acids were separated by GLC on a 6 ft 3 mm glass column packed with 6% Lac 728 on 80 to 100 mesh DMCS Chromosorb W. The oven temperature was 190° C and the helium carrier gas flow rate was 50 ml/min.

Specific gravity of alcohol-benzene extracted wood was determined by water immersion and tracheid lengths were measured after maceration. The ray volume, number of rays per unit area and the number of cells per ray were measured by preparing enlarged prints of tangential sections exposed through graft paper and counting the proportion of squares occupied by rays, the number of ray cells per print and number of cells per ray.

References

- BARTON, G. M. 1967. Differences in phenolic extracts from healthy Douglas-fir roots and those infected with *Poria weirii*. *Can. J. Bot.* 45: 1545.
- 1969. A new C-methyl flavanone glycoside from Douglas-fir roots. *Can. J. Chem.* 47: 869.
- BORMANN, F. H., in: KOZŁOWSKI, T. T. (Ed.) 1962. *Tree growth*. New York: Ronald Press 237.
- CAMPBELL, J. R., SWAN, E. P., WILSON, J. W. 1963. Comparison of wood and growth zone resinous extracts in Douglas-fir. *Pulp and Paper Mag. Canada* 66: T248.
- DADSWELL, H. E., HILLIS, W. E. in: HILLIS, W. E. (Ed.) 1962. *Wood Extractives and their Significance to the Pulp and Paper Industry*. New York. N. Y.: Academic Press 36.
- ERDTMAN, H. 1958. *Biochemistry of Wood*. Proceedings of the Fourth International Congress of Biochemistry, Vienna. Vol. II. Symposium II. New York: Pergamon Press 8.
- KIMLAND, B., NORIN, T., DANIELS, P. J. L. 1968. The constituents of the "Pocket Resin" from Douglas fir *Pseudotsuga menziesii* (Mirb.) Franco. *Acta Chem. Scand.* 22: 938.
- GARDNER, J. A. F., BARTON, G. M. 1960. The distribution of dihydroquercetin in Douglas-fir and Western larch. *For. Prod. J.* 10: 171.
- HANCOCK, W. V. 1937. The distribution of dihydroquercetin and a leucoanthocyanidin in a Douglas-fir tree. *For. Prod. J.* 7: 333.
- HEMINGWAY, R. W., HILLIS, W. E. 1969. A microanalytical method for the determination of dihydroquercetin in wood. *J. of Chromatog.* 43: 250.
- HERGERT, H. L., GOLDSCHMID, O. 1958. Biogenesis of heartwood and bark constituents 2. A new taxifolin glucoside. *J. Org. Chem.* 23: 700.
- HILLIS, W. E., HUMPHREYS, F. R., BAMBER, R. K., CARLE, A. 1962. Factors influencing the formation of phloem and heartwood polyphenols. *Holzforsch.* 16: 114.
- 1968. Chemical aspects of heartwood formation. *Wood Sci. Technol.* 2: 241.
- KENNEDY, R. W., WILSON, J. W. 1956. Variation in taxifolin content of a Douglas-fir stem exhibiting target ring. *For. Prod. J.* 6: 230.
- KRAHMER, R. L., HEMINGWAY, R. W., HILLIS, W. E. 1970. The cellular distribution of lignans in *Tsuga heterophylla* wood. *Wood Sci. Technol.* 4: 122.
- LANNER, R. M. 1961. Living stumps in the Sierra Nevada. *Ecology* 42: 170.
- SCHULTZ, R. P., WOODS, F. W. 1967. The frequency and implications of intraspecific root-grafting in Loblolly pine. *For. Sci.* 13: 226.
- SQUIRE, G. B., SWAN, E. P., WILSON, J. W. 1967. Intra-increment variation in Douglas-fir flavonoids by new technique. *Pulp and Paper Mag. Canada* 69: T431.
- WELLWOOD, R. M. 1955. Sapwood-heartwood relationships in second growth Douglas-fir. *For. Prod. J.* 5: 108.

(Received March 17, 1970)

R. W. HEMINGWAY and W. E. HILLIS,
Physiology and Microstructure Section
Division of Forest Products
C.S.I.R.O.
P.O. Box 310
Melbourne, Australia